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Chromatid Segregation at Anaphase Requires the *barren* Product, a Novel Chromosome-Associated Protein That Interacts with Topoisomerase II

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Summary

We have isolated a Drosophila gene, *barren* (*barr*), required for sister-chromatid segregation in mitosis. *barr* encodes a novel protein that is present in proliferating cells and has homologs in yeast and human. Mitotic defects in *barr* embryos become apparent during cycle 16, resulting in a loss of PNS and CNS neurons. Centromeres move apart at the metaphase–anaphase transition and Cyclin B is degraded, but sister chromatids remain connected, resulting in chromatin bridging. This phenotype is similar to that described in TOP2 mutants in yeast. Barren protein localizes to chromatin throughout mitosis. Colocalization and biochemical experiments indicate that Barren associates with Topoisomerase II throughout mitosis and alters the activity of Topoisomerase II. We propose that this association is required for proper chromosomal segregation by facilitating the decatenation of chromatids at anaphase.

Introduction

During cell division, it is essential that each cell receives a complete set of chromosomes. For proper segregation of chromosomes to occur, a number of events must first take place. After DNA replication, the chromosomes must be properly condensed. Chromosomes then become attached to spindle microtubules at kinetochores. A chromosome correctly attached is thought to be aligned at the metaphase plate by the balanced tension on the two linked, yet oppositely oriented, kinetochores (Nicklas, 1988; McIntosh, 1991; Li and Nicklas, 1995). In addition to the role of the spindle apparatus and associated motors, the chromosomes themselves play an important role in facilitating chromosome segregation. Indeed, the ability of these chromosomes to be aligned at the metaphase plate under tension is thought to require the cohesion of sister chromatids. This cohesion is released at anaphase to allow for the proper segregation of sister chromatids (for review, see Miyazaki and Orr-Weaver, 1994).

Linkages between sister chromatids have been postulated either indirectly through the presence of associated chromosomal proteins and/or directly through the DNA itself. It has been suggested that chromatid separation requires the degradation of specific protein(s) acting as a "glue." If an inhibitor of the mitosis-specific ubiquitin-dependent proteolytic system (methyl-ubiquitin) or a competitor (an N-terminal cyclin fragment containing the destruction box) is added to a Xenopus cell-free system, chromatid separation is prevented (Holloway et al., 1993; Morin et al., 1994). These observations have led to the idea that the ubiquitin-dependent degradation system acts first on a set of substrates to mediate chromatid separation and then upon the cyclins to mediate exit from mitosis. Mutations in *fizzy* prevent both of these processes (Dawson et al., 1995; Sigrist et al., 1995). In addition, mutations in *three rows* (D'Andrea et al., 1993; Philp et al., 1993) and *pimples* (Stratmann and Lehner, 1996; A. V. P., unpublished data) delay mitosis 15 at metaphase, preventing sister-chromatid separation. The data suggest that Pimples and Three Rows are required to release the cohesion between sister centromeres, thereby allowing centromere separation and initiation of chromatid segregation (Stratmann and Lehner, 1996).

In addition to proteins acting as "glues," catenation has also been proposed to contribute to sister-chromatid cohesion. The replication of chromosomal DNA leads to the catenation of strands of sister chromatids. The resolution of this catenation by Topoisomerase II is thought to occur at two steps during the cell cycle: S phase (Murray and Szostak, 1985) and the metaphase–anaphase transition. This explains the requirement for DNA Topoisomerase II activity in yeasts and in frog extracts (Uemura et al., 1987; Holm et al., 1989; Shamu and Murray, 1992). Furthermore, injection of inhibitors of Topoisomerase II into Drosophila syncytial embryos prevented chromosome segregation at anaphase (Buchenau et al., 1993). More importantly, anaphase bridging has been shown in TOP2 mutants of S. pombe, indicating a requirement for Topoisomerase II in chromatid decatenation in mitosis (Holm et al., 1985; Uemura et al., 1987). In vitro, Topoisomerase II is a rather unspecific enzyme, tangling or disentagling DNA depending on the DNA concentration (Kreuzer and Cozzarelli, 1980). Given this, it is likely that its activity in vivo is guided or controlled by other factors or proteins. These factors or proteins may play a role at various stages of the cell cycle, i.e., during S phase, during chromosome condensation, and during anaphase in chromosome segregation.

Here, we show that mutations in *barr* cause a failure of chromosomes to segregate correctly at anaphase, resulting in massive anaphase bridging. *barr* encodes a novel, conserved protein that localizes throughout chromosomes during mitosis. Since Barren associates with Topoisomerase II and affects its activity, we propose that Barren functions in chromatid decatenation during the metaphase–anaphase transition.

Results

Mutations in *barr*, a Complementation Group at 38B, Affect Nervous System Development

The *barr* gene was identified in a screen designed to isolate mutations that affect the development of the

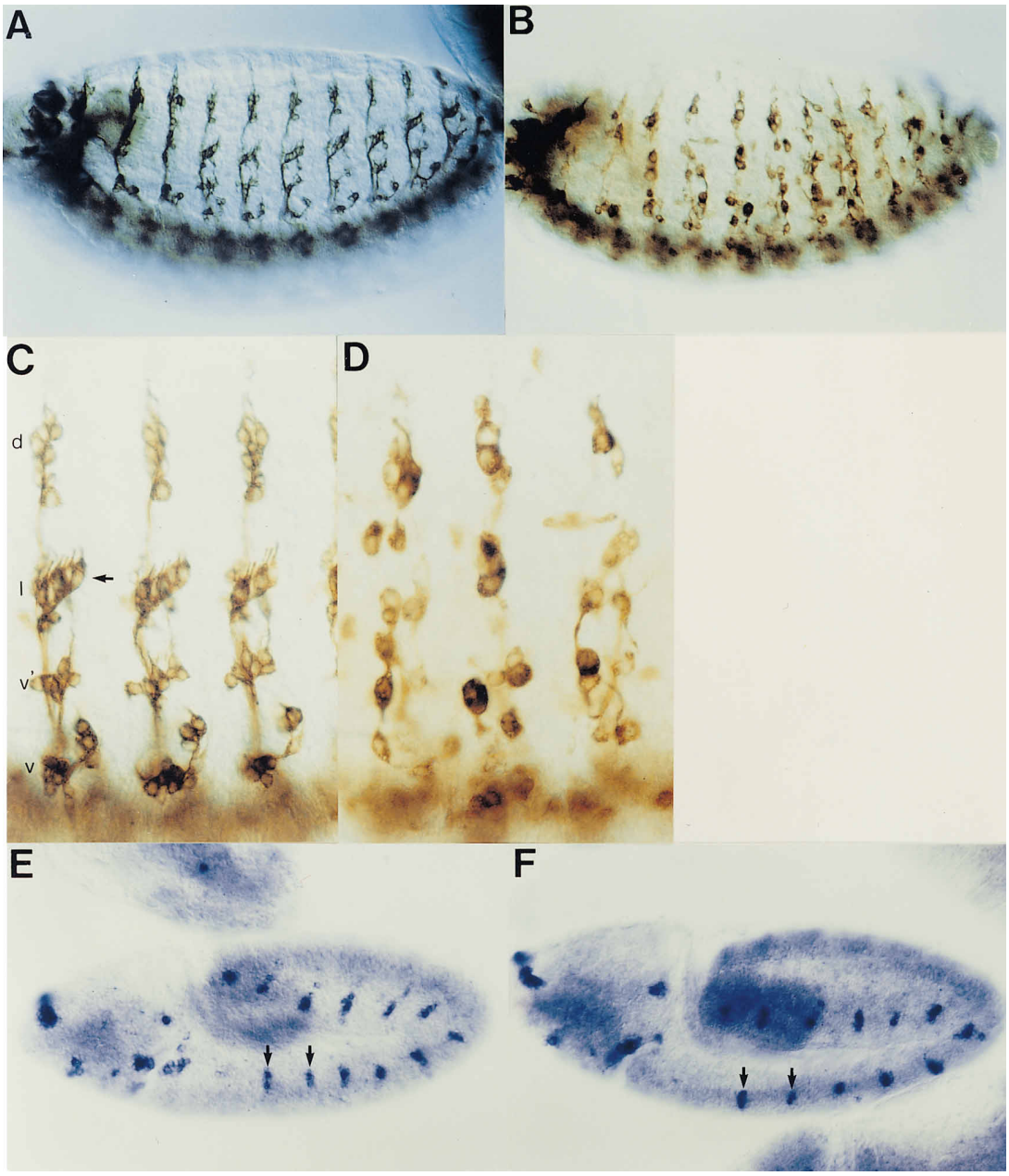


Figure 1. *barr* Mutant Embryos Exhibit Severe PNS Defects

(A) Lateral view of a stage 15 wild-type embryo stained with MAb 22C10 to reveal various clusters of peripheral neurons.

(B) A similar view of a *barr* embryo stained with MAb 22C10 shows a severe reduction in the number of neurons and lack of organization of the clusters.

(C) A magnified view of three abdominal segments of (A) indicating the neuronal clusters: d, dorsal; l, lateral; v' and v, ventral. Arrow indicates the position of five lateral chordotonals.

(D) A magnified view of three abdominal segments of (B). The mutants display a total disarray and loss of PNS neurons and an increase in their size.

(E) A wild-type stage 8 embryo hybridized with *atonal* cDNA showing the expression in chordotonal sensory-organ precursors (SOP).

(F) A similar stage *barr* mutant embryo does not show noticeable difference in expression of *atonal*, suggesting that chordotonal SOPs are born in *barr* mutants. Arrows indicate the *atonal* positive chordotonal SOPs.

peripheral nervous system (PNS) (see Figures 1A–1D). We isolated seven P-element enhancer-detector insertions that cause similar phenotypes (Kania et al., 1995) and fail to complement a small deficiency *Df(2L)TE38A-1* (38A6–38A7;38B6–38C1). All P-element insertions map at 38B1–38B2, except *P8/2*, which maps at 38B3–38B4. Complementation tests indicate that the *P8/2* insertion is in a gene encoding a kinesin-like protein currently being studied by several laboratories (Luke Alphey, Margaret Fuller, Pedro Ripoll, and Doug Ruden, personal

communications; David Glover, unpublished results). These tests also indicate that the P-element insertions in strains *P3/10*, *P139/3*, *P140/14* and *P140/28* affect *barr* only; that *P48/5* affects *barr* and the gene for a kinesin-like protein; and that *P49/12* affects *barr,* the kinesin-like protein gene, and a third complementation group, *TE7*, identified by Doug Ruden (personal communication).

To ascertain whether the P-element insertions are responsible for the mutant phenotypes, the P elements were excised. All P elements that affect *barr* only were revertible, whereas the others could not be reverted, suggesting rearrangements. These experiments also allowed us to recover an imprecise excision for *P3/10, barr^{L305}*, which behaves as a null allele of *barr* (see below).

All strains homozygous for *barr* mutations were embryonic lethal, and their embryos showed a severe loss of PNS neurons and a significant increase in size of remaining neurons (Figures 1A–1D). Mutant embryos of strain *P8/2* in which the gene for a kinesin-like protein is affected show only a decrease in the number of neurons. On the basis of the phenotypic analysis and the complementation data, we conclude that 38B1–38B4 contains at least three essential genes, two of which independently affect the development of the PNS: *barr* and a gene encoding a kinesin-like protein.

barr Embryos Arrest in Cycle 16

The loss of neurons in *barr* embryos could be attributed to the following: lack of sensory organ precursor (SOP) formation, transformation of neuronal cells into nonneuronal support cells, or failure of SOPs to divide properly. To determine whether SOPs are specified properly in *barr* embryos, we determined whether SOPs of the lateral chordotonal neurons are present, since these neurons are absent in *barr* embryos (Figures 1A–1D). In situ hybridizations with a chordotonal SOP marker (Jarman et al., 1993) show similar expression patterns in wild-type and *barr* embryos (Figures 1E and 1F), suggesting that the SOPs of lateral chordotonal neurons are born. To establish whether PNS neurons are transformed into lineage-related support cells, we stained *barr* embryos with a glial marker (Vaessin et al., 1991) and a general PNS marker (Bellen et al., 1992). These stainings revealed a severe decrease in the total number of PNS cells (data not shown). As shown in Figure 1, this decrease in number of PNS cells is associated with an increase in size of remaining cells, suggesting a failure of cell division (Salzberg et al., 1994). To assess whether the number of ectodermal cells was reduced, we visualized all nuclei with a DNA stain and noticed a 50% reduction of the total number of nuclei in the ectoderm of stage 13 embryos when compared to wild-type embryos (data not shown), suggesting that *barr* embryos fail to complete mitosis 16. This failure leads to morphological defects that are most easily observed in PNS and CNS, as these cells undergo more cycles than ectodermal cells.

barr Is Required for Complete Segregation of Chromosomes

The developmental defects of the nervous system suggest a role for *barr* in cell cycle progression. We therefore carried out immunostaining of *barr^{L305}* embryos undergoing mitosis 16 to examine spindle morphology, cyclin degradation, and cytokinesis. Cells in the dorsal and ventral epidermis of *barr* embryos (Figures 2A and 2B) enter mitosis 16 normally and undergo chromosome condensation and congression to the metaphase plate like wild-type embryos. Similarly, metaphase–anaphase transition begins correctly both by the initiation of chromosome movement and by the degradation of cyclin B (inset to Figure 2C). However, in the majority of anaphase figures, chromatids do not resolve as they do in wild type (arrows in Figure 2D) but remain interlocked to form extensive chromatin bridges (arrows in Figure 2B). Subsequently, the unresolved chromatids decondense without segregation and cytokinesis is attempted (arrows in Figure 2C), the plasma membrane being pinched in toward the chromatin bridge. Hence, *barr* is necessary for correct chromatid segregation but not cell cycle progression out of mitosis.

Centromere Separation Occurs Normally in *barr* Mutant Embryos

To examine whether centromere separation can occur in cells of *barr* embryos, we stained embryos undergoing mitosis 16 with anti-GAGA factor serum (Raff et al., 1994). This serum stains centromeric heterochromatin and, in anaphase figures, labels two areas on chromosomes, close to the spindle poles, where the centromeres lie (Figure 3A). In *barr* embryos we see centromere separation to the poles in some cells (arrows), but in others centromeres remain clustered over the central mass of chromatin (arrowhead) (Figure 3B). We also used the dodecasatellite probe to examine behavior of the centromeric region of chromosome 3 (Carmena et al., 1993; Sigrist et al., 1995). The inset to Figure 3B shows that third-chromosome centromeres (arrows) can indeed undergo extensive separation. These observations argue that incomplete separation of chromatids in *barr* embryos is not due to a failure of centromere separation but rather to some other separation or segregation defect.

Isolation of *barr*

To isolate *barr,* genomic sequences flanking the P elements were plasmid rescued and used to screen cDNA and genomic libraries. The longest cDNA obtained is 2.5 kb, and its 5′ end is 70 bp downstream of *P3/10*. In addition, sequence comparisons of genomic and cDNA sequences identified a 120 bp intron, 40 bp downstream of the start of the cDNA, just prior to the ATG start codon. PCR and sequence analyses did not reveal additional introns (Figure 4A). The cDNA probably corresponds to *barr*, as in situ hybridization experiments show that transcripts were almost absent in strain *P3/10* and absent in *barr^{L305}* embryos.

To demonstrate that we cloned *barr*, the *barr* cDNA was inserted under the control of a heat-inducible promoter into pCaSpeR-hs. Five different transgenic chromosomes were crossed into the *barr^{L305}/CyO* flies. These flies were maintained in a cycling incubator at 25°C and received a 1 hr heat shock (37°C) every 6 hr. A significant number of *y w, P{CaSpeR-hs-barr}; barr^{L305}/barr^{L305}* flies

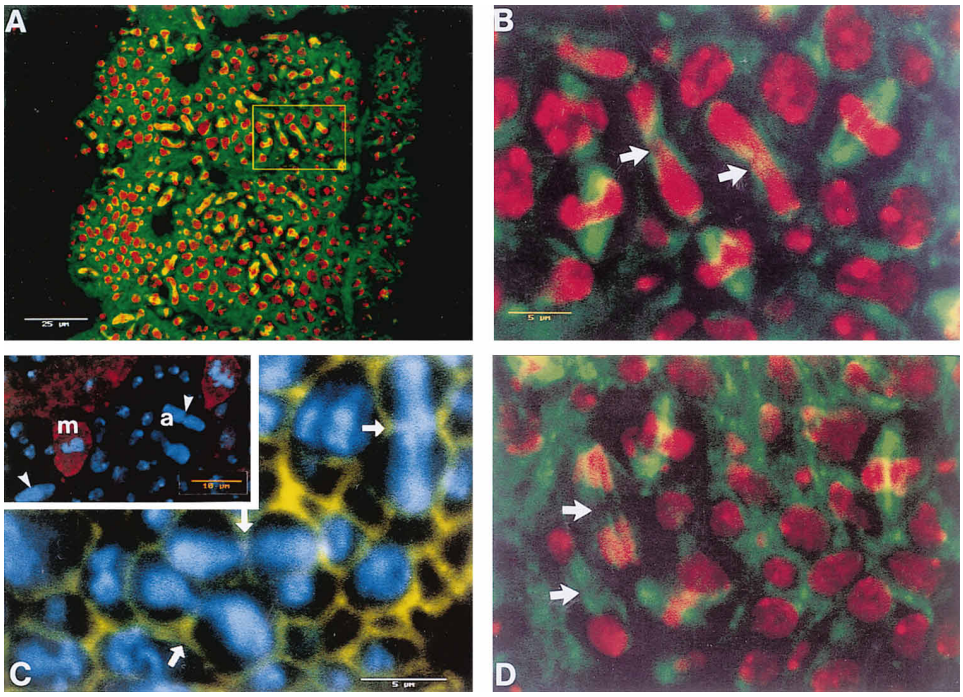


Figure 2. Barren Is Required for Chromosomal Segregation

barr embryos (A–C), and their wild-type siblings (D), undergoing mitosis 16 in the ventral and dorsal epidermal tissues. (A), (B), and (D) show views of an embryo stained with YL1/2 (a rat monoclonal serum that recognizes tyrosinated α-tubulin; green) and propidium iodide (red) to visualize DNA. (C) and the inset show views of similar embryos stained with propidium iodide (blue) and rabbit sera recognizing cyclin B (red in inset) and spectrin (yellow in main panel). Cells enter mitosis 16 following the normal pattern (A) and pass through the metaphase–anaphase transition (m, metaphase; a, anaphase) where cyclin is degraded (arrowheads in inset to [C]). However, although anaphase is initiated, the two chromosome complements cannot be disentangled from one another. (B) shows an enlargement of the boxed area on (A) and shows two such affected cells (arrows). The chromatids do not resolve and remain interlocked, forming extensive chromatin bridges rather than separating cleanly as chromatids do in wild-type embryos (arrowheads in [D]). Although chromatid separation is not completed, cytokinesis is initiated (arrows in [C]) but fails.

emerged in two independently established transgenic lines. As we never observed non-CyO flies in the *barr*^{L305}/*CyO* (*barr*^{L305} causes embryonic lethality), these observations demonstrate that we have cloned the *barr* gene.

barr Encodes a Novel Protein with Yeast and Human Homologs

The nucleotide sequence of the *barr* cDNA contains an open reading frame (ORF) of 736 aa (predicted 81 kDa). Sequence homology searches identified two ORFs: a 747 aa human (HSORF 007) and a 728 aa yeast ORF (SCYBL097W) (Figure 4B) with unknown functions. The Barren protein shows 27% or 21% identity (I) and 47% or 45% similarity (S) with the human and yeast ORF, respectively. The Drosophila and human proteins have 3 highly conserved domains: one toward the amino terminus 47% I and 77% S (102–135 aa) and two toward the carboxyl terminus with 50% I and 67% S (619–643 aa) and 49% I and 63% S (699–734 aa). There are 14 phosphorylation consensus sites for casein kinase, 11 for protein kinase-C, 3 for cAMP- and cGMP-dependent protein kinase, and one putative nuclear localization sequence (KKsahlnanRRaK) (Dingwall and Laskey, 1991). Finally, numerous PEST sequences found in rapidly degraded proteins are also present in Barren (Rogers et al., 1986). We conclude that Barren and its homologs share several key domains and identify a novel family of proteins.

barr Is Expressed in Mitotically Active Cells

In situ hybridization experiments show that *barr* is expressed in the wild-type embryo in a pattern similar to other genes essential for cell cycle progression (data not shown; Lehner and O'Farrell, 1989, 1990; Richardson et al., 1993). We did not observe *barr* in endoreplicating tissues, which suggests that it does not play a role in the S and G1 phases. Similar experiments carried out on *barr* mutant embryos show that the transcripts observed prior to stage 10 are at least partly of maternal origin. The decreased levels of transcripts in stage 8 mutant embryos suggest that zygotic transcription is initiated prior to this stage. Since developmental stages 10 and 11 correspond to mitotic cycles 15 and 16, we infer that the absence of zygotic *barr* transcripts in stage 10 *barr* mutant embryos causes the division failure observed in cycle 16.

A single 2.6 kb transcript is present at all stages except in imagos where the transcript seems shorter (data not shown). Zygotic expression peaks between 6 and 12 hr of development and mRNA is present at low levels in first, second, and third instars and at moderately low levels in pupae and adults.

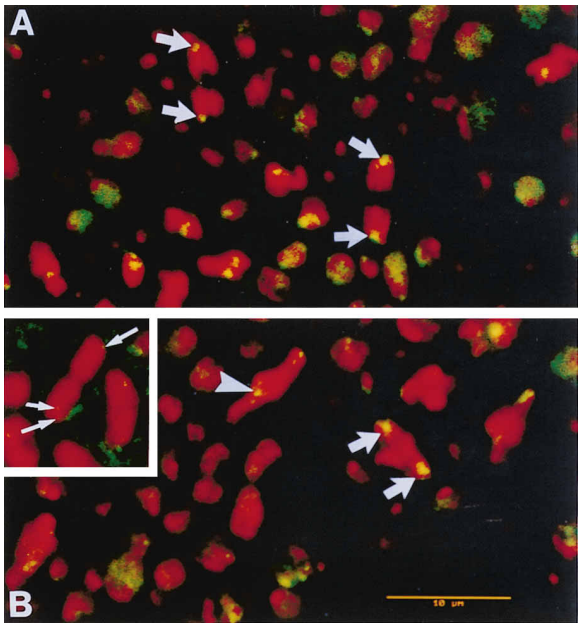


Figure 3. *barr* Mutations Do Not Prevent Centromere Separation

barr embryos (B) and their wild-type siblings (A) undertaking mitosis 16 in the ventral epidermis were stained with propidium iodide to visualize DNA (red) and an antibody that recognizes the GAGA protein (green in main panels, overlap with red gives yellow). In addition, some embryos were hybridized with a DNA probe containing the dodecasatellite sequences shown to be localized specifically near the centromere of chromosome 3 (green in inset to [B]). The GAGA serum stains centromeric heterochromatin and in wild-type anaphase figures (arrows in [A]) predominantly stains the areas of anaphase figures close to the spindle poles, where the centromeres lie. In *barr* embryos (B), we see centromere separation to the poles in many cases (arrows), but not all (arrowhead). Using the dodecasatellite probe, we observe that the centromeres (arrows) of chromosome 3 can undergo extensive separation in at least some cases.

Barren Protein Localizes to Chromatin in Mitotically Active Cells

To determine the cellular and subcellular localization of the Barren protein, we raised polyclonal antibodies against the entire ORF encoded by the cDNA. The antibodies recognized a 97 kDa protein in in vitro–translated and bacterially expressed protein lysates (Figure 5A, lanes 1 and 2). On Western blots of individual, wild-type, stage 14 embryos, the antibodies recognized a 97 kDa protein band and two bands of lower molecular weight (MW) (Figure 5A, lane 3). The intensities of the 97 kDa band and the lower MW bands were greatly reduced in wild-type stage 16 embryos, when compared to stage 14 embryos (compare in Figure 5A, lanes 3 and 4). Stage 16 mutant embryos did not show the 97 kDa protein band (Figure 5A, lane 5). In addition, the lower MW bands are also greatly reduced in Western blots of single mutant stage 16 embryos. Hence, we propose that the lower MW bands represent degradation products of the maternally contributed or maternally and zygotically contributed Barren protein in mutant and wild-type embryos, respectively.

Immunohistochemical staining of wild-type embryos showed that Barren protein is present in all cells during early development, and we did not observe immunohistochemical staining in stages 15–16 null mutant embryos.

The subcellular distribution of Barren in mitotic domains of mitosis 14 was characterized using confocal microscopy (Figure 5B). During interphase, some Barren protein is diffusely distributed throughout the cytoplasm and little or no protein is present in the nucleus. However, as chromosomes condense during prophase (p) and metaphase (m), Barren staining colocalizes with DNA staining. Throughout prophase, metaphase, and anaphase (a), the Barren staining persists on chromatin, but in late telophase (t), the staining of chromatin begins to disappear. Hence, Barren is associated with chromatin throughout the process of chromatid segregation.

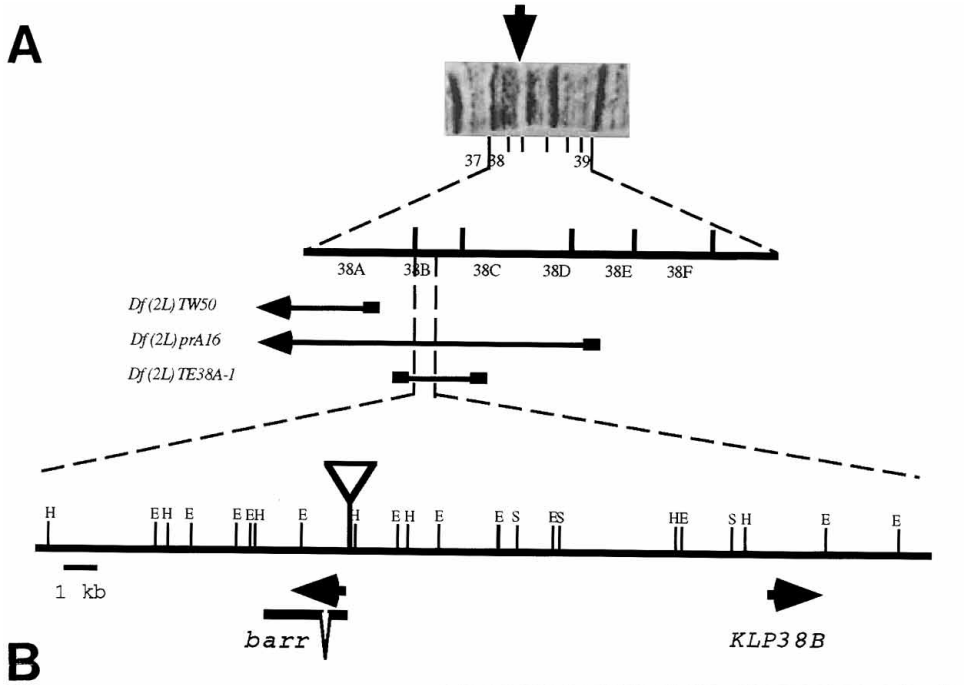
Barren Protein Colocalizes with Topoisomerase II during Mitosis

It has been proposed that the final rounds of decatenation during metaphase–anaphase transition might be carried out by Topoisomerase II (TOPO II), allowing chromosome segregation (Holm, 1994). Since *barr* embryos display chromosome bridging in anaphase, we suspected that Barren might function by modifying TOPO II activity. Using a monoclonal antibody that recognizes Drosophila TOPO II (Swedlow et al., 1993) (Figure 6A) and anti-Barren serum (Figure 6B), we stained wild-type embryos undergoing mitosis 14. TOPO II and Barren proteins colocalize during prophase (p), metaphase (m), anaphase (a), and telophase (t). However, although TOPO II associates with chromatin during interphase, Barren does not.

To determine whether TOPO II localization is dependent on *barr*, we stained $barr^{L305}$ embryos undergoing mitosis 16 with anti-TOPO II (Figures 6C and 6D). Although the chromatids cannot separate, TOPO II localizes to the metaphase plate where the tangled chromatin lies (arrowheads in Figures 6C and 6D). This indicates that although Barren may be necessary for the function of TOPO II during chromatid segregation, it is not required for localization of TOPO II to chromatin during mitosis.

Barren and Topoisomerase II Interact In Vitro and In Vivo

To determine whether Barren and TOPO II form a protein complex, we carried out immunoprecipitation experiments with anti-Barren antibodies. Embryonic extracts (0–12 hr old) were immunoprecipitated with anti-Barren antibody and resolved by SDS–PAGE. The blots were first probed with anti-Barren antibody to demonstrate that this antibody can immunoprecipitate Barren under stringent washing conditions (Figure 6E, lanes 1 and 2). Barren was below detectable levels in immunodepleted extracts as compared to embryonic extract prior to immunoprecipitation (Figure 6E, compare lanes 3 and 4). Immunoblotting with anti-TOPO II antibody identified TOPO II in anti-Barren immunoprecipitated complexes (Figure 6F, lanes 1 and 2) and embryonic extracts (lane 3). TOPO II was absent from immunoprecipitated complexes obtained with preimmune sera under identical conditions (Figure 6F, lane 4). These data suggest that



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Dm  ---------- ---------- ----------MTLP RLETPLRRSAVGSYQ EGVSRMLTPPNDDEA ERREARRRTL--LQQ   47
Hs  RRRQGKMGPPGPALP ATMNNSSSETRGHPH SASSPSERVFPMPLP R-KAPLNIPGTPVL- ED-----FPQNDDEK ERLQRRRSRVFDLQF   83

Dm  HHRSSTL------ES IE--------DNETI KN----CLELYNGNK VSKDNAWNLMLIDSL ANL-LDHHHKRMSNF KMAGSSLEASSKVYG  118
Hs  STDSPRLLASPSSRS IDISATIPKFTNTQI TEHYSTCIKLSTENK ITTKNAFGLHLIDFM SEI-LKQKDTEPTNF KVAAGTLDASTKIYA  172
Sc  ---------- --------MMANFEE ------WIKMATDNK INSRNSWNFALIDYF YDLDVLKDGENNINF QKASATLDGCIKIYS   61

Dm  LRVDSIYLDAMRISA GLSARTLTDKQINAA EDDDGPQGEQATGEG QDSAQ-------QAA KEA--APKPKRQ--K KPISTVTKNRETLN-  196
Hs  VRVDAVHADVYRVLG GLGKDAPSLEEV--- -------EGHVADGSA TEMG---------TT KK---AVKPKK---K HLHRTIEQNINNLNV  238
Sc  SRVDSVTTETGKLLS GLAQRKTNGAS-N-G DDSNGGNGEGLGGDS DEANIEIDPLTGMPI SNDPDVNNTRRRVYN RVLETTLVEFETIKM  149

Dm  SRLDTAPLQDPVPGK LNSTCGASINASNRL MHNILPSFDSELRLR TTYNFWNSEESTEEV -----QDHTTL---- --NAEMEQWPATSLM  275
Hs  SEADRKCEIDPMPQK ---TAASFDECSTAG VF--LSTLHCQ-DYR SELLFP-SDV----- -----QTLST----- ---GEPLELPELGCV  303
Sc  KELDQELIIDPLFKK ---ALVDFDEGGAKS L--LLNTLNIDNTAR V--IFDASIKDTQNV GQGKLQRKEEELIER DSLVDDENEPSQSLI  232

Dm  S----TNLMRKLLPH A--ERSNLRPLHTGY IITSA--PNPKSANE KAAEVVQDEDHDEGL DNADDVCVNKISMAF DINAECEPMPDLDGP  357
Hs  EM---TDLKAPLQQC A--EDRQICPSLAGF QFTQ---WDSETHNE SVSALVDKFKKN--- ---DQV--------F DINAEVDESDCGDFP  371
Sc  STRNDSTVNDSVISA PSMEDEILSLGMDFI KFDQIAVCEISGSIE QLRNVVEDINQ---- -AKDFIE-------- NVNNRFDNFLTEEEL  309

Dm  -PPLVLEVDSNELEE LTA----EE-QMVIN NCRRLRKQTEFIE-- --DLRPVDGNSKL-- -EYSYRP--MDQISQ FWAGPSHWKFK-RTR  431
Hs  DGSLGDDFDANDEPD HTAVGDHEEFRSWKE PCQVQSCQEEEMISLG DGDIRTMCPLLSMKP GEYSYFS--PRTMS- MWAGPDHWRFR-PRR  457
Sc  QAAVPDNAEDDSDGF DMG--MQQEL-CYPD ENHDNTSHDEQDD-- --DNVNSTTGSI--- FEKDLMAYFDENLNR NWRGREHWKVRNFKK  389

Dm  PRSTFSQTNG----Q VDTQPIRTQRAKKSA HLNANRRAKALDYGN VTENFFQQLD----- --TTIRQRKANFQ-K --KWDPRKLILPTKF  507
Hs  KQDAPSQSE------ -----NKKKSTKKDF EIDFEDD---IDF-D V---YFRKTKA----- --ATILT-KS--TLE NQNWRATT--LPTDF  518
Sc  ANLVNKESDLLEETR TTIGDTTDKNTTDDK SMDTKKKHKQKKVLE I--DFFKTDDSFEDK VFASKGRTKIDMPIK NRKNDTHYL-LPDDF  476

Dm  ELDPDLFFKYESAPS IKLSKRAG-EPDSDE GGDLGIDMDADMHHD DDND------QELF- --------NNEHFTD AVPANV--SVIAAIA  579
Hs  NYNVDTLVQLHLKPG TRLLKMA--QGHRVE TEHY-EEIE-DYDYN NPND------TSNFC PGLQAADSDDEDLDD LFVGPVGNSDLSPY-  597
Sc  HFSTDRITRLFIKPA QKMSLFSHRKHTRGD VSSGLFEKSTVSANH SNNDIPTIAD-EHF- WADNYERKEQEEKEK EQSKEV-GDVVGG--  561

Dm  AEQAAEASMMNVSAG EIGLTQ-MNATC-NN --TVFEIGTEFEGAP SQVAKVIVPFAKRAK VIDMKNLKK----SC NSLIQKQLLNAVPEE  661
Hs  PCHPPKTAQQNGDTP E---AQGLDIT---- --TYGE--SNLVAEP QKVNKIEIHYAKTAK KMDMKKLK----QSM WSLL--TALSGKEAD  670
Sc  ALD--NPFEDDMDGV DFN--QAFEGTDDNE EASVKLDLQDDEDHK FPIRENKVTYSRVSK KVDVRRLKKNVWRSI NNLIQ--EHDSRKNR  645

Dm  TIPSHP-KKKGEHYS KGFASFQQVYQKLP- DL---LTTKMSDSLS PSVAFYAVLHLANDL KLRLIPQEDLEDFQI RQVLD          736
Hs  AEANH--REAGKEAA LAEVADEKMLSGLTK DLQRSLPPVMAQNLS IPLAFACLLHLANEK NLKLEGTEDLSDVLV RQG-D          747
Sc  EQSSNDSETHTEDES TKELKFSDIIQGIS- KM---YSDDTLKDIS TSFCFICLLHLANEH GLQITHTENYNDLIV NYE-DLATTQAAS   728
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Figure 4. Map and Sequence Comparison of *barr* and Its Homologs

(A) The 38B region. The arrow indicates the position of the P-element insertions *P3/10*, *P139/3*, *P140/14*, *P48/5*, and *P49/12*. A 33 kb genomic region was isolated by a combination of plasmid rescue and chromosome walking. The *P3/10* transposon insertion site is indicated in the genomic map by a triangle. The genetic data suggested that *barr* is in close proximity to *KLP38B* that has been independently cloned by the laboratories of Minx Fuller, Pedro Ripoll, Doug Ruden, Luke Alphey, and David Glover (personal communications). *KLP38B* cDNA maps 14 kb from *barr* and is transcribed in the opposite orientation. Restriction sites are: H, HindIII; R, EcoRI; S, SalI.

(B) Sequence alignment between Barren (Dm) and its human (Hs) and yeast (Sc) homologs. Amino acids shown in bold are either conserved among all three proteins or Barren and another homolog.

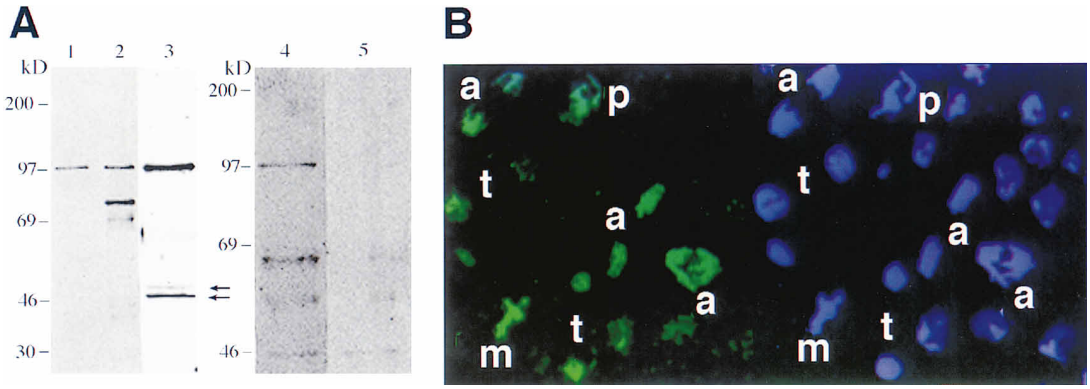

Figure 5. Localization of Barren in Proliferating Cells during Mitosis

(A) Single embryos of *barr*$^{+}$ and *barr*^{L305} were analyzed on an 8% SDS–polyacrylamide gel followed by Western blotting. Lane 1, the anti-Barren antiserum identified a single protein band migrating at 97 kDa in in vitro–translated lysates. Lane 2, expression of Barren in bacteria also produces a 97 kDa band and two other smaller MW bands. Lanes 3 and 4 each contain single wild-type stage 14 and stage 16 embryos, respectively. The antiserum recognizes a 97 kDa band and two lower MW bands of 46 and 48 kDa. The amount of Barren protein has decreased in stage 16 embryos. Lane 5, most bands including the 97 kDa band are absent or reduced in stage 16 *barr* null embryos suggesting that the 46 and 48 kDa bands are degradation products of Barren.

(B) Mitotic figures in early mitosis 14 domains stained with anti-Barren antibody (green) and propidium iodide (purple). During prophase (p), metaphase (m), and anaphase (a) Barren staining persists on chromatin, but as telophase (t) proceeds the staining of chromatin starts to fade away.

TOPO II and Barren are part of a protein complex. These complexes do not contain proteins like β-tubulin and histones, as established by Western blotting. Reciprocal immunoprecipitations with four different antibodies raised against full-length or domains of TOPO II were unsuccessful due to their inability to immunoprecipitate TOPO II.

To further establish whether Barren and TOPO II interact, we produced a recombinant GST-Barren fusion protein and immobilized it on glutathione-agarose resin. The immobilized GST alone or GST-Barren were incubated with embryonic extract. Immunoblotting revealed the presence of TOPO II following elution of GST-Barren bound proteins after extensive washing and elution with up to 100 mM NaCl. TOPO II was not present in the eluate of GST-resin (data not shown).

To determine whether the two proteins can interact in vivo, we carried out a yeast two-hybrid interaction assay. We constructed fusions of Barren and TOPO II with both the GAL4 DNA-binding domain (using pAS2-CYH2) and the GAL4 activation domain (using the pACT2) (Durfee et al., 1993). Each of the single transformants grows on the appropriate single deficient media (Figure 6G, sectors a and b, c and d) and does not activate the transcription of the β-galactosidase reporter gene (Figure 6H, sectors a–d). The pAS2-CYH2-*barr* containing strain was transformed with pACT2-*topo II* DNA, and the pAS2-CYH2-*topo II* containing strain was transformed with pACT2-*barr* DNA. Double transformants containing pAS2-CYH2-*barr* and pACT2-*topo II* (Figure 6G, sector e) or pAS2-CYH2-*topo II* and pACT2-*barr* (Figure 6G, sector f) were able to grow on medium that lacked His, Leu, and Trp showing that the HIS gene is induced. In addition, these colonies express β-galactosidase (Figure 6H, sectors e and f, respectively). Thus, the yeast two-hybrid interaction assay further established that the two proteins interact.

Barren Activates Topoisomerase II Activity

Given that three independent assays indicate that Barren interacts with TOPO II, we investigated whether Barren protein alters TOPO II activity. Barren protein was affinity purified from 0 to 16 hr embryos using anti-Barren antibody covalently linked to activated Sepharose. The eluate was highly enriched for Barren protein and had no detectable levels of TOPO II as detected by Western analysis and silver staining (data not shown). This Barren eluate was used in a DNA relaxation assay in the presence and absence of TOPO II. As shown in Figure 6I, small amounts of the Barren eluate strongly activate Topoisomerase II activity, whereas higher protein concentrations inhibit TOPO II activity. Note that at lower concentrations of the Barren protein eluate, all supercoiled DNA is converted to topoisomers (Figure 6I, lanes 3 and 4). However, as the concentration of the Barren eluate increases, we observe first weak, then strong inhibition of TOPO II activity (lanes 6 and 7 with 100 and 200 ng of Barren eluate). The Barren eluate also contains an activity that nicks a minor fraction of the supercoiled DNA. This activity increases with increasing amounts of Barren eluate and perhaps affects TOPO II activity.

As the Barren eluate activates TOPO II, we wanted to establish whether the nicking activity and TOPO II–promoting activity are due to the same protein and whether these activities could be blocked by purified anti-Barren antibodies. As shown in Figure 6J (lane 4), no nicking activity was observed when purified anti-Barren antibodies were added to DNA. The Barren eluate, in the absence of anti-Barren antibody, had some nicking ability (lane 5) that was not blocked by anti-Barren antibody (lane 1, 5 ng; lane 2, 10 ng; lane 3, 50 ng). This suggests that this minor nicking activity is due to a contaminating protein in the Barren eluate. Alternatively, this activity may be due to a degradation product

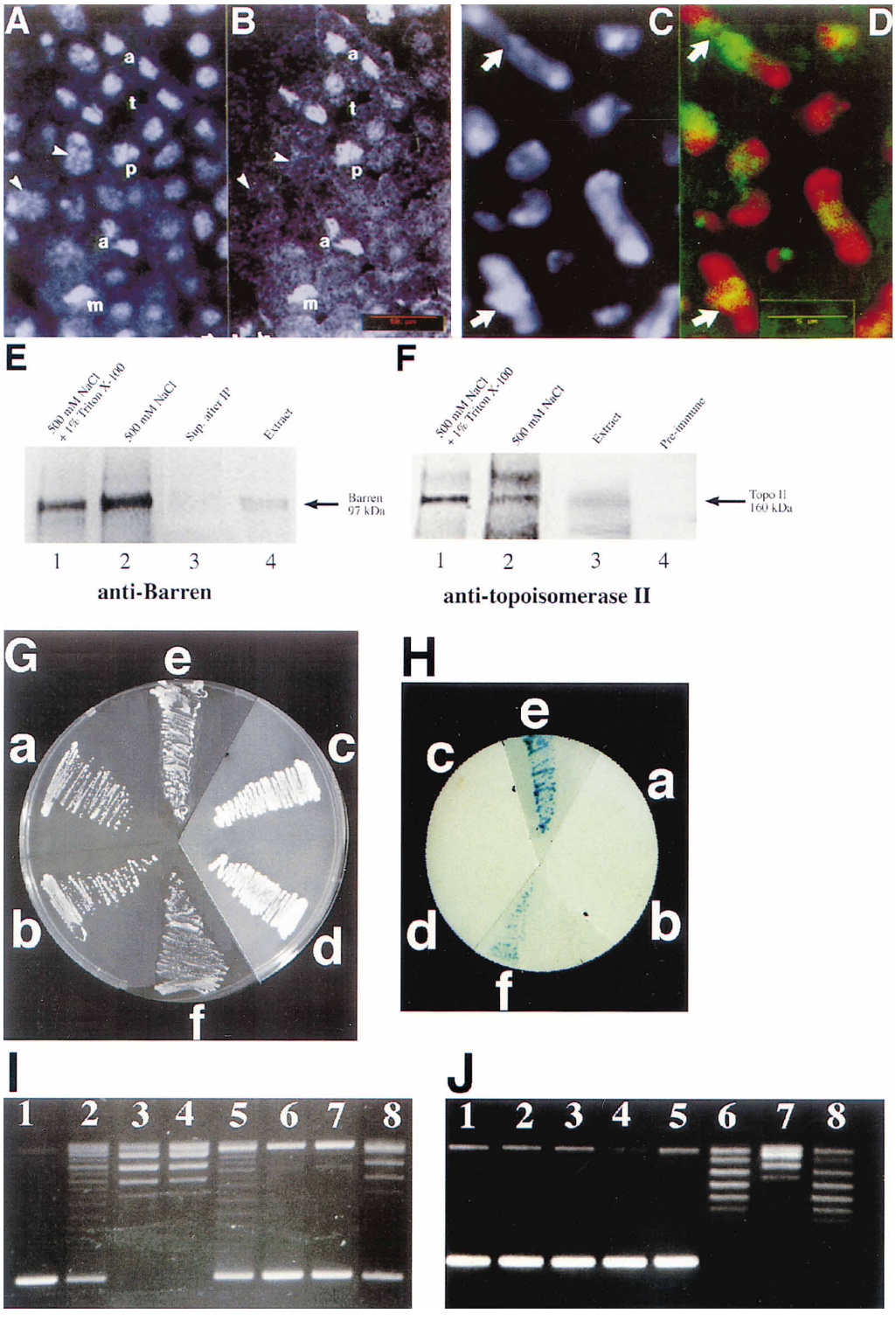

Figure 6. Barren Colocalizes, Interacts with, and Activates TOPO II

(A–D) Wild-type embryos at mitosis 14 (A and B) and *barr*^{L305} embryos at mitosis 16 (C and D) were stained with anti-TOPO II ([A], green in [D]), anti-Barren (B) and propidium iodide ([C], red in [D]) to visualize DNA. Both TOPO II (A) and Barren (B) are associated with mitotic figures from prophase (p) through metaphase (m), anaphase (a) to telophase (t). Although TOPO II is associated with chromatin in interphase, Barren is not (compare arrowheads). TOPO II (green, Figure 7D) still accumulates on elongated anaphase figures (arrows) even in the absence of Barren protein.

(E) Embryonic extracts (0–12 hr old) immunoprecipitated with anti-Barren antibody. The washing conditions used after immunoprecipitations are indicated. The immunoprecipitates were resolved by SDS–PAGE. Immunoblotting with anti-Barren antibody demonstrates that Barren (97 kDa with arrow) is immunoprecipitated (lanes 1 and 2). Barren is not present in the supernatant after immunoprecipitation (lane 3) but is present in extracts before immunoprecipitation (lane 4).

of Barren or a Barren activity that is not blocked by the anti-Barren antibody.

To determine whether the TOPO II–promoting activity in Barren eluate was due to Barren, we carried out the experiments shown in Figure 6J, lanes 6–8, using anti-Barren antibody. Lane 6 shows topoisomers produced from supercoiled DNA incubated with TOPO II. Lane 7 shows a reaction identical to that in lane 6, except that 30 ng of Barren eluate was added. Note the shift toward more relaxed topoisomers induced by the Barren eluate. Lane 8 shows the same reaction as in lane 7 in the presence of 50 ng of anti-Barren antibody. The activation of TOPO II activity due to Barren eluate as shown in lane 7 was completely blocked. We conclude that Barren eluate contains an activity that strongly enhances the ability to relax supercoiled DNA by TOPO II, and that this activity can be blocked specifically by anti-Barren antibody. The anti-Barren antibody itself has no activity in the presence or absence of Topoisomerase II and in the absence of Barren protein eluate. These data suggest that the Barren protein promotes the relaxation activity of Topoisomerase II in vitro.

Discussion

Mutations Affecting PNS Development Permit Isolation of Cell Division Genes

A previous screen for mutations that affect the development of the PNS led to the isolation of mutations that cause a decrease in total number of PNS neurons and a concomitant increase in size of remaining neurons (Salzberg et al., 1994). A similar phenotype was observed in *barr* mutant embryos (Kania et al., 1995). Salzberg et al. (1994) suggested that the giant neuron phenotype was caused by defects in cell division. The phenotype of *barr* mutants is consistent with this hypothesis as the embryos fail to proceed beyond mitosis 16. The first 13 nuclear division cycles of the syncytial embryo are driven by maternal components. If the maternal components are relatively stable, they may be sufficient for development to third-instar larvae (Gatti and Baker, 1989). In other mutants, lack of zygotic transcripts may result in a mutant phenotype in the early postblastoderm cycles, e.g., *string* (Edgar and O'Farrell, 1989). If cycles 14 and 15 proceed normally, a dramatic phenotype is not seen in most tissues, except in PNS and CNS where divisions beyond cycle 16 require zygotic transcription, e.g., *barr* mutants. In addition to *barr*, we have identified another gene at 38B that affects PNS development and encodes a kinesin-like protein. Mutants of this gene also lack PNS neurons and display mitotic defects.

barr Encodes a Conserved Protein

The Barren protein is conserved from yeast to man and is likely to play a role in cell cycle progression in other species as well. No data exist on the roles of these proteins in other organisms. The presence of PEST sequences suggests that Barren can be rapidly degraded. The lethal phases of mutations in *barr*, *fizzy* (Dawson et al., 1995; Sigrist et al., 1995), *three rows* (D'Andrea et al., 1993; Philp et al., 1993), and *pimples* (Stratmann and Lehner, 1996) suggest that the respective protein products are degraded rapidly. The degradation of Barren protein may be important in permitting cells to differentiate as they leave the cycle. However, our initial immunostaining of wild-type embryos with anti-Barren antibody suggests that degradation may not occur until the end of mitosis.

Anaphase Defects in *barr*

Mutations in *barr* prevent effective separation of sister chromatids during anaphase. The progression of the mitotic cycle up to anaphase does not appear to be affected. Chromosome condensation and congression onto the metaphase plate occurs, and this is followed by chromosome movement toward the poles at anaphase. This suggests that the spindle and associated molecular motors are functioning correctly in the mutant. Moreover, the kinetochore would seem not to be affected in the mutant since it allows normal attachment of the chromosomes to the spindle, permitting them to move to the metaphase plate. Furthermore, the kinetochore seems able to signal chromosome alignment, a mechanism monitored by tension (Ault and Rieder, 1994; Li and Nicklas, 1995), that triggers anaphase. Anaphase appears to be initiated correctly both by chromosome movement and the degradation of Cyclin B with the appropriate timing. This suggests that the mutation does not grossly affect the ubiquitin-dependent proteolytic system required for anaphase onset.

Our studies using anti-GAGA antibodies indicate that

(F) Barren immunoprecipitates were resolved on SDS–PAGE and immunoblotted with anti-TOPO II, demonstrating the presence of TOPO II (160 kDa with arrow, lanes 1 and 2). The same band is present in embryonic extract alone (lane 3). TOPO II was not detected in complexes immunoprecipitated with preimmune serum under identical conditions (lane 4).

(G and H) Results of the yeast two-hybrid interaction assay. (a) pAS2-CYH2-*barr* grown on SC-Trp, (b) pAS2-CYH2-*topo II* grown on SC-Trp, (c) pACT2-*barr* grown on SC-Leu, (d) pACT2-*topo II* grown on SC-Leu, (e) pAS2-CYH2-*barr* and pACT2-*topo II*, and (f) pAS2-CYH2-*topo II* and pACT2-*barr* grown on SC-His,-Leu,-Trp. Single transformants in sectors (a)–(d) were negative for β-galactosidase activity. Only double transformants in sectors (e) and (f) were positive for β-galactosidase activity.

(I) Effect of Barren protein eluate on TOPO II activity. Lane 1 shows control 300 ng of supercoiled pBR322 DNA without TOPO II or Barren protein eluate. Lanes 2–8 contain 300 ng DNA, 4 units of TOPO II and 1, 10, 20, 50, 100, 200 ng and no Barren protein eluate, respectively.

(J) Affinity-purified anti-Barren antibody blocks the Barren-induced activation of TOPO II activity. All lanes contain 300 ng of pBR322 DNA. Lanes 1–3 contain, in addition to DNA, 30 ng of Barren protein eluate and 5, 10, and 50 ng of anti-Barren antibody, respectively. The Barren protein eluate has weak nicking activity that is not blocked by anti-Barren antibody. Lane 4 contains DNA and 30 ng of anti-Barren antibody and no Barren protein eluate. The antibody has no nicking activity, and this lane is similar to a lane that contains DNA alone (see lane 1 of Figure 6I). Lane 5 contains DNA and 30 ng of Barren protein eluate in the absence of anti-Barren antibody. Lane 6 contains DNA and 4 units of TOPO II. Lane 7 contains 4 units of TOPO II and 30 ng Barren protein. Note the enhanced activity of TOPO II. Lane 8 contains 4 units of TOPO II, 30 ng of Barren protein eluate, and 50 ng of anti-Barren antibody. Note that the enhanced activity caused by Barren protein eluate is completely blocked.

the centromeric regions of the chromosomes are able to separate. Thus, many aspects of metaphase–anaphase transition, including chromatid separation at the kinetochores, are taking place correctly. However, sister chromatids do not separate and appear to remain linked at multiple sites along the length of the chromosomes.

Sister-chromatid cohesion has been postulated to result from the intertwining of sister DNA molecules and/or a hypothetical cohesive protein at the centromere (reviewed in Miyazaki and Orr-Weaver, 1994). The failure of chromatids to separate could be caused by a defect in either of these mechanisms. However, the fact that we frequently observe separation of centromeric regions in *barr* suggests that the mitotic defect results from chromosome arms remaining attached along their length, suggesting that decatenation of replicated chromosomes is not taking place. This led us to consider the possibility that the Barren protein is regulating the activity of TOPO II, noting that centromere separation is still observed in *topoisomerase II* mutants in S. pombe (Funabiki et al., 1993). To our knowledge, *topoisomerase II* mutants in Drosophila have not been identified. However, inhibition of TOPO II prevents complete chromatid separation in syncytial embryos (Buchenau et al., 1993). Even in the absence of *barr* function, TOPO II localizes to chromatin and is concentrated at the site of tangled DNA on the metaphase plate. Hence, Barren does not seem to be required for localization of TOPO II to the chromosomes. Interestingly, TOPO II localization studies carried out by Swedlow et al. (1993) have shown that the enzyme exists in different pools. One of these pools seems necessary for chromosomal condensation, whereas another pool seems to function at the time of chromosome segregation, suggesting different functions for these enzyme pools (Swedlow et al., 1993). Hence, Barren may modify the function of TOPO II by acting as a positive regulator for chromosomal segregation.

The need to resolve the DNA of newly replicated chromatids arises from the topological difficulties in replicating a linear chromosomal DNA molecule from multiple origins. The *barr* defect could therefore arise in the process of DNA replication. If so, this must be a type of error that would escape the checkpoint mechanisms that monitor completeness of DNA replication, as mutant cells not only enter mitosis but also proceed correctly to metaphase. These data strongly suggest that the defect is at anaphase per se. Furthermore, the absence of Barren in tissues undergoing rounds of endoreplication also argues against a requirement for the protein during S phase. The failure of decatenation of replicated chromatids may not be of consequence to the structure of polytene chromosomes, or there may be tissue-specific isoforms of Barren-like proteins that can supply this function. Our finding that the Barren protein colocalizes with TOPO II and associates with condensed chromosomes until the end of mitosis suggests that it is a chromosomal protein that plays a direct role in regulating the terminal cycles of decatenation of chromosomal arms. Our observations are consistent with this model, as immunoprecipitations, affinity chromatography using GST-Barren, and yeast two-hybrid assays indicate that Barren and TOPO II interact. In addition, biochemical assays show that eluate of affinity-purified Barren protein strongly enhances TOPO II activity and that this activity can be blocked with purified anti-Barren antibody. It will be of future interest to search for other components that regulate chromosome decatenation, providing a route toward understanding the molecular components of the decatenation process during mitosis.

Experimental Procedures

Immunohistochemistry, Immunofluorescence, and Confocal Microscopy

Embryos were collected, dechorionated, treated with taxol, fixed in 4% freshly prepared paraformaldehyde, and devitellinized in methanol as previously described in Philp et al. (1993). The following primary antibodies were used: MAb 22C10 (1:200; Zipursky et al., 1985); anti-Prospero (1:200; Vaessin et al., 1991); anti-Couch Potato (1:5000; Bellen et al., 1992); Rb271 anti-Cyclin B (1:500; Whitfield et al., 1989); YL1/2 anti-α-Tubulin (1:500; Kilmartin et al., 1982); anti-Spectrin (1:250; Byers et al., 1987); anti-GAGA (1:500; Raff et al., 1994); and anti-TOPO II (MAb P2G3, 1:10; Swedlow et al., 1993). Images were captured using a Bio-Rad MRC 600 laser-scanning confocal microscope.

Isolation of the *barr* cDNA

The genomic sequences flanking the *3/10*, *140/14*, and *139/3* P-element insertions were recovered by plasmid rescue (Wilson et al., 1989). All of the genomic phages hybridizing to the *barr* cDNA were analyzed by PCR to verify the presence of intronic sequences. The primer sequences were 5′-CTGTCGATTTGGGATCACAGT-3′ and 5′-CGACTGGGGTGAGGATATCTGT-3′ from the 5′ and 3′ ends of *barr* cDNA, respectively.

Generation of Antibodies to Barren and Western Analysis

The full-length *barr* cDNA was cloned into the pET-28a(+) expression vector (Novagen) to make a His-tag fusion protein. The induced protein was resolved by electrophoresis and excised from the gel. The protein was eluted in PBS and injected into rabbits. The immune serum was used for Western analyses at 1:3000 and for immunohistochemistry at 1:2000.

Immunoprecipitation

For immunoprecipitation, embryonic extracts were prepared by homogenizing 0–12 hr old embryos in immunoprecipitation buffer (IPB) (25 mM HEPES [pH 7.5], 100 mM NaCl, 1 mM$CaCl_2$, 1 mM $MgCl_2$, 0.5% Triton X-100, 1 mM PMSF, 5 μM leupeptin, 1 μM pepstatin, 0.3 μM aprotinin) using a Dounce homogenizer. The homogenate was filtered through Nytex mesh to remove debris and centrifuged at 20,000 $\times$ g followed by another centrifugation step at 100,000 $\times$ g for 1 hr at 4°C. The supernatant was cleared of floating lipid layer and diluted with IP buffer containing 10 mg/ml BSA (IPB). The extract was first cleared with a 50% slurry of Protein A-Sepharose (PAS) in IPB and incubated with anti-Barren antibody or preimmune serum for 2 hr at 4°C with slow tumbling, followed by addition of PAS, and continued for another 3 hr. The immunoprecipitated complexes were pelleted by brief centrifugation and washed as follows: two washes in IPB, one wash in IPB with 300 mM NaCl, one wash in IPB with 500 mM NaCl, one wash in IPB with 500 mM NaCl and 1% Triton X-10, one wash with IPB and a final wash with 10 mM Tris-HCl buffer (pH 7.5). The complexes were suspended in SDS–sample buffer and boiled for 5 min and resolved by SDS–PAGE followed by Western blotting as described above. The anti-TOPO II antibodies were used at 1:10,000 dilution (Whalen et al., 1991).

Two-Hybrid Interaction Assay

pAS2-CYH2-*barr* and pAS2-CYH2-*topo II* and pACT2-*barr* and pACT2-*topo II* were constructed by PCR amplification of complete ORFs of *barr* and *topo II*. Yeast strain HF7c (Feilotter et al., 1994) was transformed with the above plasmids. All yeast manipulations

were as described in Durfee et al. (1993). Single plasmid transformants were checked for their growth on appropriate single selection plates, as well as on double selection plates. Following sequential transformations of the plasmids in various combinations on media lacking His, Leu, and Trp, His^+ colonies were tested for β-galactosidase activity.

Affinity Chromatography with Immobilized Anti-Barren Antibody

To purify the Barren protein, we prepared an affinity matrix to which anti-Barren antibody was covalently linked. The purification of anti-Barren antibody was achieved by using One-Spin plus HiTrapQ Method (Pharmacia). This antibody preparation was then linked to CNBr-activated Sepharose 4B (Pharmacia), according to instructions of the manufacturer. This antibody-matrix was washed with several changes of low pH (pH 4.5) and high pH (pH 9.0) buffers and finally equilibrated with the lysis buffer (50 mM HEPES [pH 7.5], 50 mM NaCl, 50 mM KCl, 1 mM$CaCl_2$, 1 mM $MgCl_2$, 80 mM sodium β-glycerophosphate, 20 mM EGTA, 2 mM Na_2VO_4, 1 mM benzamidine, 1 mM sodium metabisulphite, 1 mM PMSF, 5 μM leupeptin, 1 μM pepstatin, 0.3 μM aprotinin. The source of the Barren protein was 0–16 hr old wild-type embryonic extract. The cleared extract was incubated with the antibody-matrix for 6 hr at 4°C. The matrix/extract suspension was loaded onto a column and washed with 100 bed volumes of the lysis buffer, followed by lysis buffer with increasing salt concentrations of up to 500 mM NaCl. The column was again equilibrated with 10 mM phosphate buffer (pH 7.2). The bound proteins were eluted with a linear pH gradient between 4.15 and 1.8 using glycine buffer. One-milliliter fractions were collected into 100 μl of 1M phosphate buffer (pH 7.2) to neutralize the pH. Each fraction was checked for the presence of Barren protein by Western analysis. The fractions having significantly higher amounts of Barren protein were pooled and concentrated to 30 μg/ml.

Relaxation Assay Using Topoisomerase II

TOPO II from Drosophila embryos was obtained from Amersham. The activity of TOPO II was monitored by ATP-dependent relaxation of supercoiled pBR322 DNA. Standard relaxation assay (20 μl) contained 300 ng of pBR322 DNA and 4 units of TOPO II. Addition of Barren protein preparation or purified anti-Barren antibody was followed by 30 min incubation at 4°C prior to the start of the reaction by the addition of DNA. The reaction was stopped by adding loading dye. Electrophoresis was carried out overnight at 2 V/cm followed by staining with ethidium bromide.

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References

Ault, J.G., and Rieder, C.L. (1994). Centrosome and kinetochore movement during mitosis. Curr. Opin. Cell Biol. *6*, 41–49.

Bellen, H.J., Kooyer, S., D'Evelyn, D., and Pearlman, J. (1992). The Drosophila Couch Potato protein is expressed in nuclei of peripheral neuronal precursors and shows homology to RNA-binding proteins. Genes Dev. *6*, 2125–2136.

Buchenau, P., Saumweber, H., and Arndt-Jovin, D.J. (1993). Consequences of TOPO II inhibition in early embryogenesis of Drosophila revealed by in vivo confocal laser scanning microscopy. J. Cell Sci. *104*, 1175–1185.

Byers, T.J., Dubreuil, R., Branton, D., Kiehart, D.P., and Goldstein, L.S. (1987). Drosophila spectrin, II. Conserved features of the α-subunit are revealed by analysis of cDNA clones and fusion proteins. J. Cell Biol. *105*, 2103–2110.

Carmena, M., Abad, J.P., Villasante, A., and Gonzalez, C. (1993). The Drosophila dodecasatellite sequence is closely linked to the centromere and can form connections between sister chromatids during mitosis. J. Cell Sci. *105*, 41–50.

D'Andrea, R.J., Stratmann, R., Lehner, C.F., John, U.P., and Saint, R. (1993). The three rows gene of Drosophila melanogaster encodes a novel protein that is required for chromosome disjunction during mitosis. Mol. Biol. Cell *4*, 1161–1174.

Dawson, I.A., Roth, S., and Artavanis-Tsakonas, S. (1995). The Drosophila cell cycle gene fizzy is required for normal degradation of cyclins A and B during mitosis and has homology to the CDC20 gene of Saccharomyces cerevisiae. J. Cell Biol. *129*, 725–737.

Dingwall, C., and Laskey, R.A. (1991). Nuclear targeting sequences: a consensus? Trends Biochem. Sci. *16*, 478–481.

Durfee, T., Becherer, K., Chen, P.-L., Yeh, S.-H., Yang, Y., Kilburn, A.E., Lee, W.-H., and Elledge, S.J. (1993). The retinoblastoma protein associates with the protein phosphatase type 1 catalytic subunit. Genes Dev. *7*, 555–569.

Edgar, B.A., and O'Farrell, P.H. (1989). Genetic control of cell division patterns in the Drosophila embryo. Cell *57*, 177–187.

Feilotter, H.E., Hannon, G.J., Ruddell, C.J., and Beach, D. (1994). Construction of an improved host strain for two hybrid screening. Nucleic Acids Res. *22*, 1502–1503.

Funabiki, H., Hagan, I., Uzawa, S., and Yanagida, M. (1993). Cell cycle-dependent specific positioning and clustering of centromeres and telomeres in fission yeast. J. Cell Biol. *121*, 961–976.

Gatti, M., and Baker, B.S. (1989). Genes controlling essential cell-cycle functions in Drosophila melanogaster. Genes Dev. *3*, 438–453.

Holloway, S.L., Glotzer, M., King, R.W., and Murray, A.W. (1993). Anaphase is initiated by proteolysis rather than by the inactivation of maturation promoting factor. Cell *73*, 1393–1402.

Holm, C., Goto, T., Wang, J.C., and Botstein, D. (1985). DNA topoisomerase II is required at the time of mitosis in yeast. Cell *41*, 553–563.

Holm, C., Stearns, T., and Botstein, D. (1989). DNA topoisomerase II must act at mitosis to prevent nondisjunction and chromosome breakage. Mol. Cell. Biol. *9*, 159–168.

Holm, C. (1994). Coming undone: how to untangle a chromosome. Cell *77*, 955–957.

Jarman, A.P., Grau, Y., Jan, L.Y., and Jan, Y.N. (1993). *atonal* is a proneural gene that directs chordotonal organ formation in the Drosophila peripheral nervous system. Cell *73*, 1307–1321.

Kania, A., Salzberg, A., Bhat, M., D'Evelyn, D., He, Y., Kiss, I., and Bellen, H.J. (1995). P-element mutations affecting embryonic peripheral nervous system development in Drosophila melanogaster. Genetics *139*, 1663–1678.

Kilmartin, J.V., Wright, B., and Milstein, C. (1982). Rat monoclonal anti-tubulin antibodies derived by using a new non-secreting rat cell line. J. Cell Biol. *93*, 576–582.

Kreuzer, K.N., and Cozzarelli, N.R. (1980). Formation and resolution of DNA catenanes by DNA gyrase. Cell *20*, 245–254.

Lehner, C.F., and O'Farrell, P.H. (1989). Expression and function of Drosophila cyclin A during embryonic cell cycle progression. Cell *56*, 957–968.

Lehner, C.F., and O'Farrell, P.H. (1990). The roles of Drosophila cyclin A and cyclin B in mitotic control. Cell *61*, 535–547.

Li, X.T., and Nicklas, R.B. (1995). Mitotic forces control a cell-cycle checkpoint. Nature *373*, 630–632.

McIntosh, J.R. (1991). Structural and mechanical control of mitotic progression. Cold Spring Harbor Symp. Quant. Biol. *56*, 613–619.

Miyazaki, W.Y., and Orr-Weaver, T.L. (1994). Sister-chromatid cohesion in mitosis and meiosis. Annu. Rev. Gen. *28*, 167–187.

Morin, N., Abrieu, A., Lorca, T., Martin, F., and Doree, M. (1994). The proteolysis-dependent metaphase to anaphase transition: calcium/calmodulin-dependent protein kinase II mediates onset of anaphase in extracts prepared from unfertilised Xenopus eggs. EMBO J. *18*, 4343–4352.

Murray, A.W., and Szostak, J.W. (1985). Chromosome segregation in mitosis and meiosis. Annu. Rev. Cell Biol. *1*, 289–315.

Nicklas, R.B. (1988). The forces that move chromosomes in mitosis. Annu. Rev. Biophys. Biophys. Chem. *17*, 431–449.

Philp, A.V., Axton, J.M., Saunders, R.D.C., and Glover, D.M. (1993). Mutations in the Drosophila melanogaster gene *three rows* permit aspects of mitosis to continue in the absence of chromatid segregation. J. Cell Sci. *106*, 87–98.

Raff, J.W., Kellum, R., and Alberts, B. (1994). The Drosophila GAGA transcription factor is associated with specific regions of heterochromatin throughout the cell cycle. EMBO J. *13*, 5977–5983.

Richardson, H.E., O'Keefe, L.V., Reed, S.I., and Saint, R. (1993). A Drosophila G1-specific cyclin E homolog exhibits different modes of expression during embryogenesis. Development *119*, 673–690.

Rogers, S., Wells, R., and Rechsteiner, M. (1986). Amino acid sequences common to rapidly degraded proteins: the PEST hypothesis. Science *234*, 364–368.

Salzberg, A., D'Evelyn, D., Schulze, K.L., Strumpf, D., Tsai, L., and Bellen, H.J. (1994). Mutations affecting the pattern of the PNS in Drosophila reveal novel aspects of neuronal development. Neuron *13*, 269–287.

Shamu, C.E., and Murray, A.W. (1992). Sister chromatid separation in frog egg extracts requires DNA tropoisomerase II activity during anaphase. J. Cell Biol. *117*, 921–934.

Sigrist, S., Jacobs, H., Stratmann, R., and Lehner, C.F. (1995). Exit from mitosis is regulated by Drosophila *fizzy* and the sequential destruction of cyclins A, B and B3. EMBO J. *14*, 4827–4838.

Stratmann, R., and Lehner, C.F. (1996). Separation of sister chromatids in mitosis requires the Drosophila *pimples* product, a protein degraded after the metaphase/anaphase transition. Cell *84*, 25–35.

Swedlow, J.R., Sedat, J.W., and Agard, D.A. (1993). Multiple chromosomal populations of topoisomerase II detected in vivo by time-lapse, three dimensional wide-field microscopy. Cell *73*, 97–108.

Uemura, T., Ohkura, H., Adachi, Y., Morino, K., Shiozaki, K., and Yanagida, M. (1987). DNA topoisomerase II is required for condensation and separation of mitotic chormosomes in S. pombe. Cell *50*, 917–925.

Vaessin, H., Grell, E., Wolff, E., Bier, E., Jan, L.Y., and Jan, Y.N. (1991). prospero is expressed in neuronal precursors and encodes a nuclear protein that is involved in the control of axonal outgrowth in Drosophila. Cell *67*, 941–953.

Whalen, A.M., McConnell, M., and Fisher, P.A. (1991). Developmental regulation of Drosophila DNA topoisomerase II. J. Cell Biol. *112*, 203–213.

Whitfield, W.G.F., Gonzalez, C., Sanchez-Herrero, E., and Glover, D.M. (1989). Transcripts of one of two Drosophila cyclin genes become localized in pole cells during embryogenesis. Nature *338*, 337–340.

Wilson, C., Pearson, R.K., Bellen, H.J., O'Kane, C.J., Grossniklaus, U., and Gehring, W.J. (1989). P-element mediated enhancer detection: an efficient method for isolating and characterizing developmentally regulated genes in Drosophila. Genes Dev. *3*, 1301–1313.

Zipursky, S.L., Venkatesh, T.R., and Benzer, S. (1985). From monoclonal antibody to gene for a neuron-specific glycoprotein in Drosophila. Proc. Natl. Acad. Sci. USA *82*, 1855–1859.

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